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(54) Title: SITE SPECIFIC LISTERIA INTEGRATION VECTORS AND METHODS FOR USING THE SAME

(57) Abstract: Site-specific Listeria integration vectors and methods for their use are provided. The subject vectors include a bacteriophage integrase gene and a bacteriophage attachment site, where in many embodiments the bacteriophage that is the source of these elements is a listeriophage. In certain embodiments, the subject vectors further include a multiple cloning site, where the multiple cloning site may further include a polypeptide coding sequence, e.g., for a heterologous antigen. The subject vectors and methods find use in a variety of different applications, including the study of Listeria species and the preparation of Listeria vaccines.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/13492

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/00, 15/09, 15/63, 15/70, 15/74  
US CL : 435/320.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
U.S. : 435/320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
Please See Continuation Sheet

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	LAUER et al. Construction, Characterization, and Use of Two Listeria monocytogenes Site-Specific Phage Integration Vectors. Journal of Bacteriology. August 2002. Volume 184, Number 15, pages 4177-4186, see entire document.	1-11
X	SCHAFERKORDT, S. et al. Vector Plasmid for Insertional Mutagenesis and Directional Cloning in Listeria spp. BioTechniques. 1995, Volume 19, Number 5, pages 720-725, see entire document.	1-9
X	FORTINEAU et al. Optimization of green fluorescent protein expression vectors for in vitro and in vivo detection of Listeria monocytogenes. Res. Microbiol. 2000, Volume 151, pages 353-360, see entire document.	1-9
Y	REITER et al. Transfer RNA genes frequently serve as integration sites for prokaryotic genetic elements. Nucleic Acids Research. 1989, Volume 17, Number 5, pages 1907-1914, see entire document.	5
A	SCOTT et al. Conjugative Transposition. Annu. Rev. Microbiol. 1995, Volume 49, pages 367-397, see entire document.	1-11

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance  
"E" earlier application or patent published on or after the international filing date  
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)  
"O" document referring to an oral disclosure, use, exhibition or other means  
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention  
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone  
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art  
"&" document member of the same patent family

Date of the actual completion of the international search

12 November 2003 (12.11.2003)

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/13492

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claim Nos.: 20, 21, 22, 24  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:  
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-11

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

**BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING**

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group 1, claims 1-11, drawn to an integration vector capable of site-specific *Listeria* genome integration.

Group 2, claim 12, drawn to a method of transforming cells using the integration vector.

Group 3, claims 13, 16-19, drawn to a *Listeria* transformed with the integration vector and cultures and compositions thereof.

Group 4, claims 14-15, drawn to a method of eliciting or boosting a cellular immune response using the *Listeria* transformed with the integration vector.

The inventions listed as Groups 1-7 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical feature of Group 1 is an integration vector capable of site-specific *Listeria* genome integration. This technical feature is anticipated by the art according to Schaeferkordt et al (BioTechniques, 19(5):720-725, 1995) that teaches an insertion site-specific vector plasmid for cloning in *Listeria* species. Inasmuch as, the technical feature of Group 1 does not define a contribution over the art, the technical feature of Group 1 is not "special" within the meaning of PCT Rule 13.2 and therefore Groups 1-7 lack unity of invention.

**Continuation of B. FIELDS SEARCHED Item 3:**

WEST, MEDLINE, EMBASE, DERWENT.

SEARCH TERMS: VECTOR, LISTERIA, INTEGRATE, PHAGE, LISTERIOPHAGE, INTEGRASE, ATT, tRNA, SITE-SPECIFIC, SPECIFIC INTEGRATION.